Development of an assay to identify pre-emergence herbicide resistance in *Alopecurus myosuroides* grass populations

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Introduction

More and more farmers need sequential herbicide applications to control black-grass with non-target-site resistance. Due to different mode of actions pre-emergence herbicides play an important and increasing role in these control strategies. Resistance to pre-emergence compounds can appear. Testing pre-emergence herbicides include many challenges compared to post-emergence herbicides. The aim of this study was to find a standard method to validate pre-emergence herbicide resistance in a practicable way for monitoring programs.

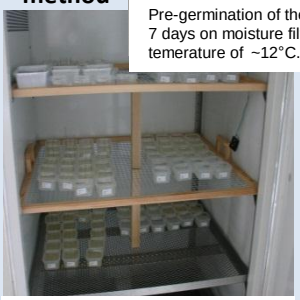
Material and methods

- Test of four different black-grass biotypes: two susceptible biotypes (sen) + two biotypes with confirmed NTSR(R)
- herbicides tested: prosulfocarb, flufenacet, chlorotoluron, metazachlor+quinmerac
- dose-response curves with five to six rates per herbicide
- use of pre-germinated seeds
- herbicide application directly after transplanting (BBCH 05-08) with a lab sprayer
- comparison of three different methods:
  - soil-based in the greenhouse
  - soil based outdoor
  - agar based in climate chambers
- validation with a field trial
- measurements of root- and shoot length on dat 21 to 28
- calculations of ED50 values and resistance factors (RF) (Streibig, 1988) and the discriminating rate for each herbicide

Pre-germination of the seeds

Pre-germination of the seeds after 5-7 days on moisture filter paper and a temerature of ~12°C.

Agar method



Climate chamber with a constant temperature of 12°C and a light period of 12 h (22,900 lux)

Sample of an agar pot, treated with 0.8 g ai/ha metazachlor+quinmerac

Herbicides	HRAC	Product name, concentration, supplier	Method	Rates [g ai/ha] [a]					
				1	2	3	4	5	6
Prosulfocarb	N	Boxer (800 g L^{-1} prosulfocarb, Syngenta Agro GmbH)	soil	200	400	2000	4000	20000	
			agar	4	40	200	2000	4000	
			field	1000	2000	4000	8000	24000	
Metaza-chlor+ quinmerac	K3+O	Butisan Top (375 g L^{-1} metazachlor, 125 g L^{-1} quinmerac, BASF SE)	soil	38	75	188	375	750	1500
			agar	0.8	1.9	3.8	7.5	18.8	75
			field	56	188	375	750	1500	
Chloro-toluron	C2	Lentipur700 (700 g L^{-1} chlorotoluron, Nufarm)	soil	210	525	1050	2100	10500	21000
			agar	21	105	525	1050	2100	10500
			field	525	1050	2100	4200	10500	
Flufenacet	K3	Cadou SC (500 g L^{-1} flufenacet, Bayer Crop Science)	soil	12.5	25	125	250	1250	
			agar	0.3	2.5	12.5	62.5	250	
			field	12.5	62.5	125	250	500	

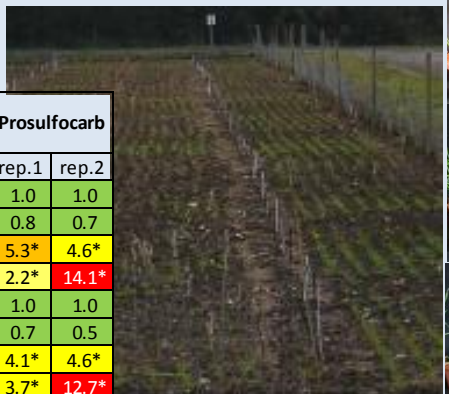
[a] All methods were conducted with a untreated control,
Underlined: Max. registered herbicide doses (Boxer: 5.0 l/ha, Butisan Top: 2.0 l/ha, Lentipur: 3.0 l/ha, Cadou: 0.5 l/ha).
Red: discriminating rates - statistically higher growth reduction of the S biotypes compared to the R biotypes.

Results

RF of each herbicide, method and biotype based on ED50 value of senH for both repetitions

		Flufenacet		Chlorotoluron		Metazachlor +quinmerac		Prosulfocarb	
Method	Biotype	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2	rep.1	rep.2
Outdoor pot	senH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	senA	0.8	0.3	2.5	0.9	1.9	0.8	0.8	0.7
	pel	1.9	1.5*	143.1*	23.2*	4.4*	4.1*	5.3*	4.6*
	Elbe	1.9	1.6*	105.7*	8.1*	9.8*	6.8*	2.2*	14.1*
Indoor pot	senH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	senA	1.0	1.4	3.9	0.5	1.8	0.9	0.7	0.5
	pel	2.6*	2.8*	219.5*	20.4*	9.1*	4.2*	4.1*	4.6*
	Elbe	5.6*	1.7*	56.0*	12.1*	13.9*	7.8*	3.7*	12.7*
Field trial	senH	1.0		1.0		1.0		1.0	
	senA	1.4		0.6		0.6		0.8	
	pel	2.3		10.3*		2.3		14.3*	
	Elbe	3.9		7.0*		1.3		15.6*	
Agar shoot	senH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	senA	1.6	1.6	6.8	2.5	1.8	0.9	0.9	1.3
	pel	1.4	1.6	262.3*	28.8*	1.9	1.8*	4.7	1.7
	Elbe	3.1	2.1	201.0*	32.0*	0.6	1.2*	5.4	2.9
Agar root	senH	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	senA	1.6	1.2	7.9	1.1	1.0	1.1	0.8	0.8
	pel	1.2	1.4	226.1*	3.2*	6.4*	2.9*	1.7	0.7
	Elbe	3.7	0.9	157.0*	3.8*	4.4*	3.2*	5.5	4.1

* significant difference in growth reduction between the R and sen biotypes



View on the field trial on 13th December. (Sowing on 25th September, herbicide application on 1st October 2014)

Pot trial with sandy loam (pH-value 6.5, organic matter content ~2%), nine seeds per pot, three repetitions, prosulforcarb, dat 21

Summary and conclusions

- **Resistance detection is possible and varies between the test methods**
- **The field trial confirmed resistance**
- **Indoor pot method is the most practical way for testing the four different pre emergence herbicides**

→ **Discriminating dosages for further monitoring are:**

2000 g ai/ha prosulfocarb (2.5 l/ha Boxer)
75+25 g ai/ha metazachlor+quinmerac (0.2 l/ha Butisan Top)
2100 g ai/ha chlorotoluron (3.0 l/ha Lentipur)
125 g ai/ha flufenacet (0.25 l/ha Cadou SC)